Studies on Nodule Bacteria. XII.

Influence of various iron compounds on the nodule bacteria of Astragalus sinicus, (Genge).

By

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The influence of twelve different iron salts representing the ferrous and ferric compounds of organic and inorganic nature viz., ferrous sulfate, ferrous chloride, ferrous carbonate, ferrous iodide, ferric sulfate, ferric chloride, ferric nitrate, ferrous lactate, ferric oxalate, ferric malate, ferric tartarate and ferric citrate, on the growth of Genge nodule bacteria was investigated.

This investigation was undertaken to ascertain the significance of iron salts on the nodule bacteria since it is well known that the presence of large amount of soluble iron especially the ferrous compounds in the paddy field is injurious to the growth of rice plants although, in general, a small amount of soluble iron salts is found.

On the other hand, the presence of a trace of iron in cultivation of micro-organisms is absolutely essential. Throne and others[5] demonstrated with nodule bacteria and B. subtilis that no growth of these organisms on the synthetic media without iron was obtained after the second or third generation.

Again, as to the stimulation by iron, many reports are found. For example, Remy and Rosing[1], and Söhngen[2] found that the colloidal iron stimulated the growth of Azotobacter and the urea bacteria; Burk[3] reported that the nitrogen fixation by Azotobacter chroococcum was stimulated by the soluble inorganic as well as organic iron salts, and noted that 50 ppm was the optimum concentration and no ill effect was observed by an increased amount; Throne[5] experimented with nodule bacteria and found both ferrous sulfate and ferric chloride stimulated the growth best at 10 ppm concentration, and the ferric iron was less harmful than the ferrous iron, and the maximum limit was 60 ppm and 40 ppm respectively.

The results obtained will be reported as follows.

Experimental.

1.) *The Genge nodule bacteria isolated in our laboratory were grown on the medium of following composition :*

K_2HPO_4	0.5 g.	$MgSO_4$	0.2 g.	NaCl	0.2 g.	$CaSO_4$	0.1 g.
$CaCO_3$	1.0 g.	KNO_3	0.5 g.	Mannitol	10.0 g.	Agar	15.0 g.
Water	1,000 cc.						

To which the various iron salts were added in the following concentration, calculated as iron:

0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.00005 per cent.

2.) Examination of the rate of growth:

With an envigorated culture suspension in 100 cc. sterile water, the agar slant was inoculated by streaking on the surface as uniformly as possible by means of the platinum needle, and incubated at 28°C. and the growth was examined on the third, fifth and seventh day, and compared with the control.

3.) Determination of pH values:

The P_H values of the media were determined by the direct method after the media were prepared.

4.) Determination of iron salts:

Since the iron salts added to the medium become insoluble and some of the ferrous compounds are transformed into the ferric, it was necessary to determine them quantitatively after the medium was prepared, and the method recommended by OSUGI and NISHIGAKI[4] was used.

Results.

1.) Influence of iron compounds on the growth of nodule bacteria.

The results obtained with the concentration of 0.1–0.0001 per cent iron salts are given in Table 1 and those of 0.05–0.00005 per cent in Table 2.

Table 1.

Influence of Iron Compounds on the Growth Nodule Bacteria. (I)

Iron compounds.	Concentration.	P_H	Rate of growth.					
			3 days.		5 days.		7 days.	
	(%)							
Ferrous sulfate.	0.1	5.82	—	—	—	—	—	—
	0.01	6.67	++	++	+++	+++	+++	++++
	0.001	—	++	++	+++	++++	++++	++++
	0.0001	—	++	++	+++	+++	++++	++++
Ferrous chloride.	0.1	5.58	—	—	—	—	—	—
	0.01	6.51	++	++	+++	+++	+++	+++
	0.001	—	+++	+++	+++	+++	++++	++++
	0.0001	—	++	++	+++	+++	+++	++++
Ferrous carbonate.	0.1	6.67	++	++	+++	+++	+++	++++
	0.01	6.67	++	++	+++	+++	++++	++++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	++	++	+++	+++	++++	++++

Table 1. (Continued.)

Iron compounds.	Concentration.	P_H	Rate of growth.					
			3 days.		5 days.		7 days.	
	(%)							
Ferrous iodide.	0.1	6.39	—	—	—	—	—	—
	0.01	6.55	—	—	+	+	++	++
	0.001	—	++	++	+++	+++	+++	+++
	0.0001	—	++	++	+++	++++	++++	++++
Ferric sulfate.	0.1	5.62	—	—	—	—	—	—
	0.01	6.51	++	++	+++	+++	+++	+++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	++	++	+++	++++	++++	++++
Ferric chloride.	0.1	6.50	+	+	+	+	++	++
	0 01	6.60	++	++	+++	+++	++++	++++
	0.001	—	+++	+++	+++	++++	++++	++++
	0.0001	—	++	++	+++	++++	++++	++++
Ferric nitrate.	0.1	6.06	++	++	++	++	++	++
	0.01	6.51	+++	+++	+++	+++	+++	++++
	0.001	—	++	++	+++	+++	+++	++++
	0.0001	—	++	++	+++	+++	++++	++++
Ferrous lactate.	0.1	6.17	—	—	—	—	—	—
	0.01	6.58	++	++	+++	+++	++++	++++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	++	++	+++	+++	+++	+++
Ferric oxalate.	0.1	6.26	+	+	++	++	+++	+++
	0.01	6.35	++	++	+++	+++	++++	++++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	++	++	+++	+++	++++	++++
Ferric malate.	0.1	6.65	+++	+++	+++	+++	++++	++++
	0.01	6.51	++	++	+++	+++	++++	++++
	0.001	—	++	++	+++	++++	++++	++++
	0.0001	—	++	++	+++	+++	++++	++++
Ferric tartarate.	0.1	5.87	—	—	+	+	++	++
	0.01	6.65	+++	+++	+++	++++	++++	++++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	++	++	++	++	+++	+++
Ferric citrate.	0.1	6.17	—	+	++	++	++	++
	0.01	6.25	+	+	++	++	+++	+++
	0.001	—	++	++	+++	+++	++++	++++
	0.0001	—	+++	+++	+++	+++	+++	++++
Control.	—	6.46	+	+	+++	+++	++++	++++

Notes: — No growth; the number of + indicates the rate of growth.

Table 2.

Influence of Iron Compounds on the Growth Nodule Bacteria. (II)

Ironc ompounds.	Concentration.	P_H	Rate of growth.					
			3 days.		5 days.		7 days.	
Ferrous sulfate.	(%) 0.05	5.65	−	−	−	−	−	−
	0.005	6.48	+	−	##	##	##	##
	0.0005	6.36	+	−	##	##	##	##
	0.00005	6.44	+	+	#	##	##	##
Ferrous chloride.	0.05	5.47	−	−	−	−	−	−
	0.005	6.48	+	−	##	##	##	##
	0.0005	6.29	+	+	##	##	##	##
	0.00005	6.32	+	−	##	##	##	##
Ferrous carbonate.	0.05	6.59	+	−	##	##	##	##
	0.005	6.48	+	#	##	##	##	##
	0.0005	6.36	+	−	##	##	##	##
	0.00005	6.55	+	+	##	##	##	##
Ferrous iodide.	0.05	6.06	−	−	−	−	−	−
	0.005	6.31	−	−	+	#	##	##
	0.0005	6.37	−	−	+	#	##	##
	0.00005	6.53	+	+	#	##	##	##
Ferric sulfate.	0.05	6.03	−	−	−	−	−	−
	0.005	6.37	−	−	#	#	##	##
	0.0005	6.29	+	−	##	##	##	##
	0.00005	6.36	+	−	##	##	##	##
Ferric chloride.	0.05	5.28	−	−	−	−	+	+
	0.005	6.27	+	+	##	##	##	##
	0.0005	6.34	+	+	##	##	##	##
	0.00005	6.34	+	+	##	##	##	##

Table 2. (Continued)

Iron compounds.	Concentration.	p_H	Rate of growth.					
			3 days.		5 days.		7 days.	
Ferric nitrate.	0.05	5.80	+	−	−	−	−	−
	0.005	6.12	+	−	+	++	++	++
	0.0005	6.31	+	−	+	++	+++	+++
	0.00005	6.34	+	−	++	+++	++++	++++
Ferrous lactate.	0.05	6.18	−	−	+	+	++	++
	0.005	6.25	+	+	+++	+++	+++	+++
	0.0005	6.31	+	−	+++	+++	+++	+++
	0.00005	6.31	+	−	++	+++	+++	+++
Ferric oxalate.	0.05	6.31	+	+	++	++	+++	+++
	0.005	6.51	+	+	+++	+++	+++	+++
	0.0005	6.39	+	+	+++	+++	+++	+++
	0.00005	6.48	+	+++	+++	+++	+++	+++
Ferric malate.	0.05	6.65	−	−	+++	+++	+++	++++
	0.005	6.65	+	−	+++	+++	+++	+++
	0.0005	6 50	+	+	+++	+++	+++	+++
	0.00005	6.53	+	−	++	+++	+++	+++
Ferric tartarate.	0.05	4.38	−	−	−	−	−	−
	0.005	5.56	+	−	+++	+++	+++	+++
	0.0005	6.13	+	−	+++	+++	+++	+++
	0.00005	6.34	+	+	++	+++	+++	+++
Ferric citrate.	0.05	5.30	−	−	+++	+++	+++	+++
	0.005	5.89	+	++	+++	+++	+++	+++
	0.0005	6.05	+	+	+++	+++	+++	+++
	0.00005	6.20	+	−	++	+++	+++	+++
Control.	—	6.38	+	+	+++	+++	+++	+++

Notes: — No growth; the number of + indicates the rate of growth.

From these results presented in Table 1 and 2, the optimum concentration of each salts for the growth is summarized in Table 3.

Table 3.

Summary of Table 1 and 2 on the Optimum Concentration of each Salt for the Growth.

Iron compounds.	Concentration. (%)	Rate of growth.	
Ferrous sulfate.	0.001	++++	++++
	0.0001	++++	++++
	0.0005	++++	++++
Ferrous chloride.	0.001	++++	++++
	0.0005	++++	++++
	0.00005	++++	++++
Ferrous carbonate.	0.01	++++	++++
	0.05	++++	++++
Ferrous iodide.	0.0001	++++	++++
	0.00005	++++	++++
Ferric sulfate.	0.001	++++	++++
	0.0005	++++	++++
Ferric chloride.	0.001	++++	++++
	0.005	++++	++++
Ferric nitrate.	0.0001	++++	++++
	0.00005	++++	++++
Ferrous lactate.	0.01	++++	++++
	0.001	++++	++++
	0.0001	++++	++++
	0.0005	++++	++++
Ferric oxalate.	0.0001	++++	++++
	0.005	++++	++++
	0.0005	++++	++++
Ferric malate.	0.01	++++	++++
	0.05	++++	++++
Ferric tartarate.	0.001	++++	++++
	0.005	++++	++++
Ferric citrate.	0.0001	++++	++++
	0.0005	++++	++++
Control.	—	++++	++++

Notes: The number of + indicates the rate of growth.

As noted in Table 3, the optimum concentration differs by the different salt, as follows :

Optimum concentration.	Names of salts.
(%) (0.05 – 0.01)	Ferrous carbonate, Ferric malate.
(0.01 – 0.0005)	Ferrous lactate.
(0.005 – 0.001)	Ferric chloride, Ferrous sulfate, Ferric sulfate.
(0.001 – 0.00005)	Ferrous chloride.
(0.0005 – 0.0001)	Ferric oxalate, Ferric citrate.
(0.0001 – 0.00005)	Ferric nitrate, Ferrous iodide.

As shown above, the highest optimum concentration was found with ferrous carbonate and ferric malate while the lowest ones were ferric nitrate and ferrous iodide. In all the cases, the addition of iron gave better growth over the control, and among them ferric chloride of 0.005 per cent and ferric malate of 0.05 per cent concentration were very effective in stimulating the growth, followed in the order by 0.0005 per cent ferric sulfate, 0.0005 per cent ferric citrate, 0.005 and 0.0005 per cent ferric oxalate, and 0.005 per cent ferric tartarate. The maximum limit for the harmful influence examined at the seventh day of growth was as shown in Table 4.

Table 4.

Maximum Concentration for the Inhibition of Growth.

Iron compounds.	Concentration.	Iron compounds.	Concentration.
	(%)		(%)
Ferrous sulfate.	0.01	Ferric nitrate.	0.001
Ferrous chloride.	0.01	Ferrous lactate.	0.05
Ferrous carbonate.	0.1	Ferric oxalate.	0.05
Ferrous iodide.	0.001	Ferric malate.	0.1
Ferric sulfate.	0.01	Ferric tartarate.	0.05
Ferric chloride.	0.05	Ferric citrate.	0.01

As shown in Table 4, ferric malate was the least harmful followed by ferrous carbonate, and ferric nitrate and ferrous iodide depressed the growth even at 0.001 per cent concentration.

From the foregoing results, ferric malate was found to be very effective in stimulating the growth and also the least harmful, and its optimum concentration was 0.01 – 0.05 per cent. The optimum concentration for other salts was as follows ; ferric chloride, 0.001 – 0.005 ; ferric oxalate, 0.0001 – 0.0005 ; ferric tartarate, 0.001 –

0.005; ferric sulfate, 0.001 – 0.005; and ferric citrate, 0.0001 – 0.0005 per cent. Ferric nitrate was least stimulating and most strongly harmful, which is followed by ferrous iodide.

In general the organic iron salts were less harmful and seemed a little better in stimulating the growth but practically no difference was found as to the injurious action.

As to the action of iron on the nodule bacteria, it was found to be different by the nature of compounds used. But as a whole, it seems that neither marked stimulation nor harmful influence is brought about by an addition of iron compounds judging from the fact that even a most harmful compound added in 0.00005 per cent concentration grew equally well as the control.

2.) Determination of soluble iron in the culture media.

Since the iron compounds change their forms in the course of preparation of media as to their solubility and state, ferrous into ferric, the determination of soluble iron was made by adding 0.05 and 0.005 per cent of each iron compound into the culture solution, sterilized and left standing for two to three days, and then filtered so that the insoluble portion is eliminated. In case of 0.05 per cent concentration, the ferrous and ferric irons were determined, and for 0.005 per cent, the whole was oxidized and determined as the ferric iron. The results are shown in Table 5.

Table 5.

Quantity of Soluble Iron found after the Media are prepared.

Iron compounds.	Soluble iron found.		
	50 mg. added.		5 mg. added.
	Fe^{II}	Fe^{III}	(Fe^{II} + Fe^{III})
	(mg.)	(mg.)	(mg)
Ferrous sulfate.	11.20	12.32	0.28
Ferrous chloride.	6.10	8.40	0.28
Ferrous carbonate.	trace	trace	trace
Ferrous iodide.	trace	trace	trace
Ferric sulfate.	—	10.12	trace
Ferric chloride.	—	1.12	0.14
Ferric nitrate.	—	2.80	trace
Ferrous lactate.	1.08	10.12	0.14
Ferric oxalate.	—	25.20	0.28
Ferric malate.	—	7.36	0.23
Ferric tartarate.	—	7.16	0.14
Ferric citrate.	—	38.08	3.36

As noted in the above table, the quantity of soluble iron decreased markedly as well as the ferrous state changed into the ferric state, and in some cases, only a trace of iron was found. Consequently the initial conditions by which the

experiments were started, did not remain in the course of experimentation. For these reasons, all the interpretation of the results were made on the basis of the initial quantity of soluble iron added.

Summary.

The influence of twelve different iron salts of organic and inorganic nature on the growth of Genge nodule bacteria was investigated. The iron salts were applied in different concentration covering the ranges of 0.05–0.00005 per cent, and the following results were obtained.

1.) The marked stimulation was obtained by ferric malate and the chlorides which were followed by ferric sulfate, ferric citrate, ferric oxalate and ferric tartarate while the others were indifferent.

2.) Ferric malate was least harmful and followed by ferrous carbonate while ferrous iodide and ferric nitrate depressed the growth.

3.) As a whole the influence of these iron salts on the growth of the nodule bacteria may be considered to be rather small as far as our results indicate.

Literature.

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